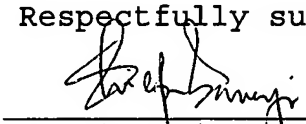
are administered a nucleic acid, rather than CD34+ cells, the cells are now specified as limited to stem cells and wherein the cells are able to differentiate into any hematopoietic cell type." The Examiner contends that those amendments consequently necessitate a new search and further consideration. Applicants respectfully disagree that a new search is necessary.

Before the amendments to claims 18, 23, 37 and 52, the recited CD34+ cells comprised a population of CD34+Thy-1+Lin- cells, as indicated by the clause "wherein said population of CD34+ hematopoietic cells includes a subpopulation of pluripotent $CD34^{+}Thy\text{-}1^{+}Lin^{-}$ cells." By amending the claims applicants limited the CD34+ cells to pluripotent hematopoietic stem cells that are $CD34^{+}Thy\text{-}1^{+}Lin^{-}$ cells. Both the term "$CD34^{+}Thy\text{-}1^{+}Lin^{-}$" and the term "pluripotent hematopoietic stem cells" were limitations in the claims before the amendments and presumably have been searched by the Examiner.[1] In any event, Applicants' amendments have narrowed, not broadened, the claims and do not present new issues requiring further consideration or search.

[1] The characteristic that these cells can "differentiate into any hematopoietic cell type" is inherent to $CD34^{+}Thy\text{-}1^{+}Lin^{-}$ cells.

The Examiner has indicated that the January 7, 2005 amendments would overcome the prior art rejections. Accordingly, applicants respectfully request that the Examiner enter those amendments, consider the foregoing remarks and allow the pending claims.

Respectfully submitted,



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PATENTS
Attorney Docket No. CENT/002 CPA RCE

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

PATENT APPLICATION

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|---|---|---|
| Applicants | : | Young et al. |
| Application No. | : | 09/237,291 Confirmation No. : 9391 |
| Filed | : | January 25, 1999 |
| For | : | EXPANDED AND GENETICALLY MODIFIED POPULATIONS OF HEMATOPOIETIC STEM CELLS |
| Group Art Unit | : | 1635 |
| Examiner | : | K. Lacourciere |

New York, New York
January 7, 2005

Hon. Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

RESPONSE TO FINAL OFFICE ACTION

This responds to the June 2, 2004 Final Office Action. Applicants file this response in accordance with a telephonic interview with the Examiner on January 5, 2005. As this response is being filed within two months of the filing of the Notice of Appeal, no fees are believed to be due. However, if a fee is due, the Director is authorized to charge it to deposit account 06-1075.

**Amendments to the Claims** are reflected in the listing of claims that begins on page 3 of this paper.

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**Remarks** begin on page 11 of this paper.

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application.

**Listing of Claims:**

1-17. (canceled)

18. (currently amended) A method for obtaining genetically modified human pluripotent hematopoietic stem cells, comprising:

a) contacting a vector comprising a polynucleotide sequence encoding a heterologous gene with a population of human ~~CD34+~~ pluripotent hematopoietic stem cells cultured with fibronectin and in the presence of an effective amount of a mpl ligand and a flt3 ligand, each ligand provided in a concentration range of about 0.1 ng/mL to about 500 ng/mL, wherein said vector is selected from the group consisting of retroviral vectors, adenoviral vectors, and adeno-associated viral vectors and wherein said ~~population of CD34+~~ human pluripotent hematopoietic stem cells ~~includes a subpopulation of pluripotent~~ are $CD34^{+}Thy\text{-}1^{+}Lin^{-}$ cells and can differentiate into any hematopoietic cell type; and

b) obtaining said modified human pluripotent hematopoietic stem cells.

19. (currently amended) The method according to claim 18, further comprising culturing the population of human pluripotent hematopoietic stem cells in the

presence of a c-kit ligand in a concentration range of about 5 ng/mL to about 200 ng/mL prior to contacting said cells with said vector.

20. (currently amended) The method according to claim 19, further comprising culturing the population of human pluripotent hematopoietic stem cells in the presence of interleukin 3 (IL-3) in a concentration range of about 5 ng/mL to about 200 ng/mL prior to contacting said cells with said vector, wherein said concentration range does not cause differentiation of the human pluripotent hematopoietic stem cells.

21-22. (canceled)

23. (currently amended) A method for obtaining genetically modified human pluripotent hematopoietic stem cells, comprising:

a) contacting a vector comprising a polynucleotide sequence encoding a heterologous gene with a population of human ~~CD34+~~ pluripotent hematopoietic stem cells cultured with fibronectin and in the presence of an effective amount of [[a]] thrombopoietin ~~ligand~~ (TPO), a flt3 ligand (FL), and interleukin 6 (IL-6), wherein the TPO, FL and IL6 are each provided in a concentration range of about 0.1 ng/mL to about 500 ng/mL, and wherein said vector is selected from the group consisting of retroviral vectors, adenoviral vectors, and adeno-associated viral vectors and wherein said ~~population of CD34+~~ human pluripotent

hematopoietic stem cells are ~~include a subpopulation of pluripotent~~ $CD34^{+}Thy\text{-}1^{+}Lin^{-}$ cells and can differentiate into any hematopoietic cell type; and

b) obtaining said modified human pluripotent hematopoietic stem cells.

24. (currently amended) The method of claim 23, further comprising culturing the human pluripotent hematopoietic stem cells in the presence of ~~an effective amount of~~ leukemia inhibitory factor (LIF) ~~wherein said effective amount is in the~~ in a concentration range of about 5 ng/mL to about 200 ng/mL prior to contacting said cells with said vector.

25. (currently amended) The method of claim 23, further comprising culturing the human pluripotent hematopoietic stem cells in the presence of ~~an effective amount of~~ interleukin 3 (IL-3) ~~wherein the effective amount is in the~~ in a concentration range of about 10 ng/mL to about 100 ng/mL prior to contacting said cells with said vector.

26. (currently amended) The method of claim 23, further comprising culturing the human pluripotent hematopoietic stem cells in the presence of a c-kit ligand in a concentration range of about 5 ng/mL to about 200 ng/mL prior to contacting said cells with said vector.

27. (currently amended) The method of claim 25, further comprising culturing the human pluripotent hematopoietic stem cells in the presence of a c-kit ligand in a

concentration range of about 5 ng/mL to about 200 ng/mL prior to contacting said cells with said vector.

28-30. (canceled)

31. (previously presented) The method according to claim 23, wherein the effective amount of TPO and FL individually is in the range of about 5 ng/mL to about 200 ng/mL and the effective amount of IL-6 is in the range of about 10 ng/mL to about 100 ng/mL.

32. (previously presented) The method according to claim 23, wherein the vector is a retroviral vector.

33. (previously presented) The method according to claim 23, wherein the heterologous gene is a marker gene.

34. (currently amended) The method according to claim 23, further comprising expanding the modified human pluripotent hematopoietic stem cells.

35-36. (canceled)

37. (currently amended) A method of transducing human ~~CD34+ hematopoietic cells including a subpopulation of~~ pluripotent $CD34^{+}Thy\text{-}1^{+}Lin^{-}$ hematopoietic stem cells, comprising:

a) obtaining a source of <u>said</u> ~~hematopoietic cells including the subpopulation of pluripotent CD34+Thy-1+Lin- hematopoietic~~ stem cells<u>, wherein said stem cells can differentiate into any hematopoietic cell type</u>;

b) culturing said cells with fibronectin and the cytokines thrombopoietin (TPO), flt3 ligand (FL), and interleukin 6 (IL-6), individually provided in the range of about 0.1 ng/mL to about 500 ng/mL;

c) infecting the cultured cells with a retroviral vector including a polynucleotide sequence encoding a heterologous gene; and

d) obtaining transduced cells wherein said gene is expressed.

38. (previously presented) The method according to claim 37, wherein the TPO, FL and IL-6 are individually provided in the range of about 5 ng/mL to about 200 ng/mL.

39. (currently amended) The method according to claim 37, further comprising culturing the cells in the presence of ~~an effective amount of~~ leukemia inhibitory factor (LIF) ~~wherein said effective amount is in the~~ <u>in a concentration</u> range of about 5 ng/mL to about 200 ng/mL.

40. (currently amended) The method according to claim 37, further comprising culturing the cells in the presence of ~~an effective amount of~~ IL-3 ~~wherein said effective amount is in the~~ in a concentration range of about 10 ng/mL to about 100 ng/mL, wherein said concentration range does not cause differentiation of the human pluripotent hematopoietic stem cells.

41. (currently amended) The method according to claim 39, further comprising culturing the cells in the presence of ~~an effective amount of~~ IL-3 ~~wherein said effective amount is in the~~ in a concentration range of about 10 ng/mL to about 100 ng/mL.

42. (currently amended) The method according to claim 37, wherein said ~~effective amount of~~ IL-6 is in the range of about 10 ng/mL to about 100 ng/mL.

43. (previously presented) The method according to claim 37, wherein the TPO is provided as a mimetic.

44-45. (canceled)

46. (previously presented) The method according to claim 37, wherein the heterologous gene is a marker gene.

47. (previously presented) The method according to claim 37, wherein the heterologous gene is a therapeutic gene.

48. (previously presented) The method according to claim 18 wherein the fibronectin is RetroNectin™.

49. (previously presented) The method according to claim 23 wherein the fibronectin is RetroNectin™.

50. (previously presented) The method according to claim 37 wherein the fibronectin is RetroNectin™.

51. (canceled)

52. (currently amended) A method for obtaining genetically modified human pluripotent hematopoietic stem cells, comprising:

a) contacting a vector comprising a polynucleotide sequence encoding a heterologous gene with a population of human pluripotent ~~CD34+~~ hematopoietic stem cells cultured with fibronectin and in the presence of an effective amount of a mpl ligand and a flt3 ligand, each ligand provided in a concentration range of about 0.1 ng/mL to about 500 ng/mL, and optionally in the presence of one or more cytokines selected from: c-kit ligand in a

concentration range of about 5 ng/mL to about 200 ng/mL, interleukin 3 (IL-3) in a concentration range of about 5 ng/mL to about 200 ng/mL, leukemia inhibitory factor (LIF) in a concentration range of about 5 ng/mL to about 200 ng/mL, and interleukin 6 (IL-6) in a concentration range of about 5 ng/mL to about 200 ng/mL, wherein said vector is selected from the group consisting of retroviral vectors, adenoviral vectors, and adeno-associated viral vectors, and wherein said stem cell can differentiate into any hematopoietic cell type; and

b) obtaining said modified human pluripotent hematopoietic stem cells.

## REMARKS

Applicants thank the Examiner for granting a telephonic interview with their representatives, Ying Li and Shilpi Banerjee, on January 5, 2005 and for her assistance during the interview. Applicants have amended the claims based on that interview, as further discussed below.

Claims 18-20, 23-27, 31-34, 37-43, 46-50, and 52 are pending in this application. Applicants respectfully request reconsideration of the application in view of the following amendments and remarks.

**The Interview**

During the January 5, 2005 interview, applicants and the Examiner focused their discussion on claim 18, which was representative of the pending claims. Claim 18 was directed to a method of genetically modifying human pluripotent hematopoietic stem cells with a viral vector, where the starting cell population was cultured in the presence of at least two cytokines – a mpl ligand such as TPO and a flt3 ligand (FL).

Applicants pointed out that, of all the references cited by the Examiner, only four of them relate to culturing hematopoietic cells in the presence of TPO and/or FL – Ku, Kobayashi, Ramsfjell, and Ohmizono. Applicants pointed out that the cells used in those references were committed progenitor cells and were at best multipotent. None of those four references taught or suggested culturing pluripotent hematopoietic stem cells in the presence of TPO and FL. Applicants explained that based on the definition in the specification (page 1, first ¶), pluripotent hematopoietic stem cells can differentiate into any hematopoietic cell type,

and are more primitive than committed progenitor cells or multipotent progenitor cells, which can differentiate only into limited hematopoietic lineages.

Applicants proposed amending the claims to require that the starting cell population be pluripotent stem cells that are $CD34^{+}Thy\text{-}1^{+}Lin^{-}$ and that can differentiate into any cell type. The Examiner agreed that the proposed amendment would overcome the outstanding prior art rejections and that she would enter it.

**Claim Amendments**

Accordingly, independent claims 18, 23, 37, and 52 are now amended to specify that the cells contacted with the viral vector are pluripotent human hematopoietic stem cells that are $CD34^{+}Thy\text{-}1^{+}Lin^{-}$ and can differentiate into any hematopoietic cell type. Support for this amendment appears in the specification at page 1, lines 4-11; and page 4, lines 27-30. Dependent claims 19, 20, 24-27 and 34 are amended to reflect the changes in the base claims.

Claims 18 and 52 are further amended to correct clerical errors – inserting the article "a" before "mpl ligand" and inserting a comma before "each ligand." Claims 23-25 and 39-42 are amended for conciseness. These amendments do not change the scope of the claims.

No new matter is introduced by the above amendments. In addition, all amendments set forth above raise no new issues that would require further consideration and/or search. Applicants submit that these amendments place the claims into condition for allowance, or at least present the rejected claims in better form for consideration on appeal, and should therefore be entered after the final rejection. 37 C.F.R. § 1.116 (a).

**Rejections under 35 U.S.C. § 103(a)**

All of the pending claims – claims 18-20, 23-37, 31-34, 37-43, 46-50 and 52 – stand rejected as being obvious over Murray, Nakahata, Hoffman, Fei or Davis, in view of Ku, Kobayashi, Ramsfjell, Ohmizono, Szilvassy, Escary, or Bodine, and further in view of Tushinski, Fletcher, Bello-Fernandez, or Hatzfeld, and Hanenburg (Nature Medicine) or Hanenburg (Human Gene Therapy). Applicants respectfully traverse in view of the claim amendments.

The pending claims are directed to methods of genetically modifying human pluripotent hematopoietic stem cells with a viral vector, where the stem cells are cultured in the presence of at least two cytokines – a mpl ligand such as TPO, and a flt3 ligand (FL). Of all the references cited, only Ku, Kobayashi, Ramsfjell, and Ohmizono relate to culturing hematopoietic cells in the presence of TPO and/or FL.

As applicants noted during the January 5, 2005 interview, none of those four references teach that a mpl ligand such as TPO and FL can support proliferation of pluripotent hematopoietic stem cells without causing differentiation.[1] Rather, those references refer to multipotent cells that give rise to a limited subset of blood cell types.

For example, Ramsfjell makes a careful distinction between multipotency and pluripotency. While noting that its TPO-cultured cells were "multipotent," Ramsfjell admits that it failed to establish that those cells had lymphoid potential (p. 5176, left col., last full ¶).

[1] Applicants also note that Ku does not teach the combined use of TPO and FL. Ku refers to the combined use of sTPOR and FL. STPOR is a soluble receptor for TPO, and is not TPO.

That reference further states that it has yet to be seen that TPO can "expand the true long term reconstituting pluripotent stem cells" (p. 5176, right col., 1st ¶; emphasis added). Likewise, Kobayashi observes that "[b]oth FL and SF produced predominantly GM colony-forming cells in synergy with TPO . . . ." (p. 427, right col.). See also in applicants' June 30, 2003 and March 8, 2004 responses to office actions.

As previously discussed, the other cited references do not teach or suggest that TPO and FL can be used to expand pluripotent hematopoietic stem cells while retaining the cells' ability to differentiate into any hematopoietic cell type.

In conclusion, applicants respectfully submit that the proposed amendments place the claims in condition for allowance. The Examiner is invited to telephone the undersigned to discuss any issues remaining in this application.

Respectfully submitted,

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